Studies on Serine Peptides. III. Synthesis of L-Seryl-L-Histidine, L-Seryl-L-Histidyl-L-Leucine and Carbobenzyloxy-Glycyl-O-Benzyl-DL-Serine Ethyl Ester

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Seryl-histidyl peptide was found in insulin by Sanger, who suggested that the peptide plays an important role in the biological action[1]. It was reported that the same peptide groups might also be contained in ribonuclease[2] and chymotrypsin[3].

With regard to synthetic studies of seryl peptides, the author reported in the previous paper[4] that the *O*–Bz*-L-serine was a useful starting material for the synthesis of seryl peptides, and *N*-Cbzo**-*O*-Bz-L-seryl-L-histidine methyl ester was synthesized in a good yield. Although the synthetic studies on histidyl peptides have been reported by Holley and Fischer[5,6] only a few simple peptides were obtained by the azide method in an unsatisfactory yield. Fischer and Whetston reported that Cbzo-glycyl-L-histidine azide was coupled with L-serine methyl ester or L-tyrosin methyl ester in the yields of 25 and 43 % of theory, respectively.

In the present work, *N*-Cbzo-*O*-Bz-L-seryl-L-histidine was coupled with leucine methyl ester in dioxane by the use of Sheehan's dicyclohexylcarbodiimide[7] to give *N*-Cbzo-*O*-Bz-L-seryl-L-histidyl-L-leucine methyl ester in 68 % yield. The free peptides, L-seryl-L-histidine and L-seryl-L-histidyl-L-leucine, were prepared from their Cbzo-*O*-benzyl derivatives by treatment with hydrogen bromide in dioxane. The esterification of *O*-Bz-L- and DL-serine was carried out in the ethanol-dry hydrogen chloride solution at low temperature. *N*-Cbzo-glycyl-*O*-Bz-DL-serine ethyl ester was obtained in 88% yield by Sheehan's method.

Acknowledgement: Low yields in the synthesis of histidyl peptides by means of azide method are possibly caused by the condensation of imidazole and acyl azide group.

This difficulty was overcome by the use of dicyclohexylcarbodiimide method. *N*(α)-Cbzo-histidine still has a zwitter ion character owing to the presence of imidazole ring, nevertheless the amino group is carbobenzoxylated, and it is difficultly soluble in usual organic solvents. On the contrary, *N*-Cbzo-*O*-Bz-L-seryl-L-histidine is not soluble in hot water but soluble in dioxane and chloroform. This fact favors the homogeneous reaction giving high yields of the reaction product.

The author tried to remove the protecting groups of the *N*-Cbzo-*O*-Bz-L-serine by using dry hydrogen bromide in glacial acetic acid according to the Ben-Ishai's method[8], but hereby *O*-acetyl-serine was obtained in place of free L-serine. In dioxane, the elimination of protective groups from peptide derivatives proceeded smoothly and free peptides were obtained in desirable yields. It is interesting that the benzyl residue in *O*-Bz-serine is not attacked in the esterification procedure but is readily cleft by the use of hydrogen bromide in dioxane. The results provide further evidences that the free hydroxy group of serine must be protected by a suitable group to obtain good results in peptide synthesis.

Experimental

***N*-Cbzo-*O*-Bz-L-seryl-L-histidine.**—*N*-Cbzo-*O*-Bz-L-seryl-L-histidine methyl ester[9] was prepared from *N*-Cbzo-*O*-B_2-L-serine and L-histidine methyl ester by Sheehan's method. Into the solution of 25 g. of the carbobenzoxy-peptide ester in 52 cc. of dioxane was added 52 cc. of aqueous solution of

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* Bz-; Benzyl-

** Cbzo-; Carbobenzyloxy-

1) *Symposium on Peptide Chemistry*, 1955 (London), p. 24.

2) C. H. W. Hirs, *Fed. Proc.*, **16**, 196 (1957), R. R. Redfield and C. B. Anfinsen, *J. Biol. Chem.*, **221**, 385 (1956). C. H. W. Hirs, W. H. Stein and S. Moore, ibid., **221**, 151 (1956).

3) J. R. Whitaker and B. J. Jandorf, *J. Biol. Chem.*, **223**, 751 (1956).

4) K. Okawa, This Bulletin, **29**, 486 (1956).

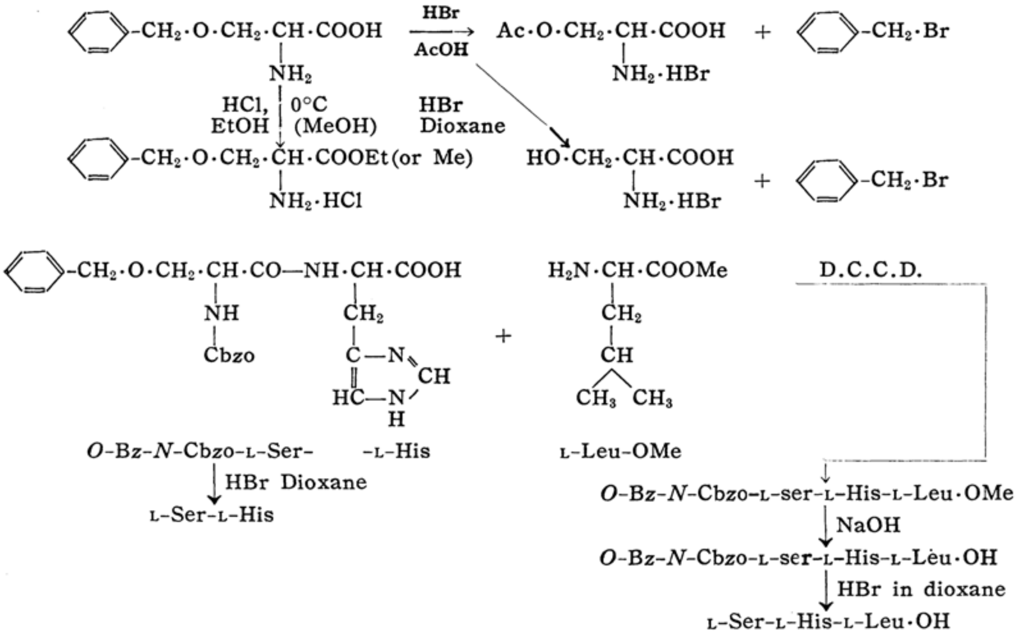
5) R. W. Holley and E. Sondheimer, *J. Am. Chem. Soc.*, **76**, 1326 (1954).

6) R. F. Fischer and R. R. Whetstone, ibid., **76**, 5076 (1954).

7) J. C. Sheehan and G. P. Hess, ibid., **77**, 1067 (1955).

8) Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

9) K. Okawa, This Bulletin, **29**, 489 (1956).



1 N-sodium hydroxide portion-wise in 20 minutes under stirring. After kept standing for 30 minutes, the reaction mixture was brought to pH 2.0 with hydrochloric acid, and washed with ethylacetate. The aqueous layer was brought to pH 4.4, and 18.0 g. of Cbzo-peptide was precipitated, m.p. 156°C. Recrystallization from methanol-ether gave 16.0 g. of pure material, m.p. 164°C. in 66.8 % yield. $[\alpha]_D^{13}=+20.7$ (10 mg/cc. AcOH)

Anal. Found: C, 60.40; H, 5.39; N, 12.04. Calcd. for $C_{24}H_{26}O_6N_4$: C, 61.80; H, 5.58 N, 12.02 %.

***N*-Cbzo-*O*-Bz-L-seryl-L-histidyl-L-leucine Methyl Ester.**—Two grams of *N*-Cbzo-*O*-Bz-L-seryl-L-histidine in 80 cc. of dioxane was coupled with 1 g. of dicyclohexylcarbodiimide in 20 cc. of tetrahydrofurane and L-leucine methyl ester in 10 cc. of tetrahydrofurane (prepared from 1.0 g. of its hydrochloride by the use of ammonia-chloroform solution[10]). After the solution was left at room temperature overnight, 0.3 cc. of acetic acid was added to remove the unchanged dicyclohexylcarbodiimide, and the urea derivative produced was filtered off. The filtrate was concentrated in vacuo, the residue dissolved in ethylacetate, the solution washed with dilute acid, alkali and water and then dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the residual precipitate was collected and washed with petroleum ether. The yield was about 2.0 g. Recrystallization from ethylacetate-petroleum ether gave 1.5 g. of carbobenzoxypeptide ester. From the mother liquor, 0.1 g. of the crystals were obtained. The total yield was 1.6 g. (64 %), m.p. 152–153°C. $[\alpha]_D^{13}=-20.14$ (10 mg./cc. EtOH)

Anal. Found: C, 62.60; H, 6.60; N, 11.61. Calcd. for $C_{31}H_{39}O_7N_5$: C, 62.73; H, 6.57; N, 11.79 %.

***N*-Cbzo-*O*-Bz-L-seryl-L-histidyl-L-leucine.**—By the usual method, 1.2 g. of the peptide ester was dissolved in 2cc. of dioxane and 2 cc. of an aqueous solution of 1 N sodium hydroxide was added portion-wise into dioxane solution and the reaction mixture was allowed to stand for 50 minutes at room temperature. When the hydrolysis was over, 1 cc. of 2 N hydrochloric acid was added into the solution, and the fine precipitate produced was filtered (1.1 g.). Recrystallization from methanol gave 0.7 g. of the carbobenzoxy-peptide in 68.7 % yield, m.p. 172–173°C.

Anal. Found: C, 61.00; H, 6.17; N, 11.99. Calcd. for $C_{30}H_{37}O_7N_5$: C, 62.17; H, 6.38; N, 12.08 %.

***O*-Ac-L-serine.**—Dry hydrogen bromide was passed into the solution of *N*-Cbzo-*O*-B_2-L-serine (1.1 g.) in 7 cc. of acetic acid for twenty minutes. After the solution was left at room temperature for 30 minutes, 70 cc. of absolute ether was added into the reaction mixture. The white precipitate produced was washed twice with dry ether. The products (0.65 g., 94.2 %) were dried in a vaccum desiccator over sodium hydroxide.

By treating this product with triethylamine in absolute ethanol, *O*-Ac-L-serine was obtained.

Anal. Found: C, 40.35; H, 5.87; N, 9.56. Calcd. for $C_5H_9O_4N$: C, 40.81; H, 6.09; N, 9.52 %.

Dioxane was used in place of acetic acid in the above procedure. The crystals of L-serine monohydrogen bromide could be obtained in 90 % yield.

L-Seryl-L-histidine Mono Hydrogen bromide.—Dry hydrogen bromide was passed into the suspension of 1 g. of *N*-Cbzo-*O*-Bz-L-seryl-L-histidine in 7 g. of dioxane at room temperature

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[10] G. Hillmann, *Z. Naturforsch*, **1**, 682 (1946).

until the concentration of hydrogen bromide reached 25 % and then the mixture was allowed to stand for an hour. In this reaction period, the carbobenzoxy-peptide was dissolved perfectly into the dioxane solution. To the reaction mixture was added dry ether and the precipitate was collected and washed with ether three times. The precipitate was likely to be slightly hygroscopic. The yield was about 1.2 g. The precipitate was dissolved in 5 cc. of ethanol and dry pyridine was added until the solution became slightly turbid (pH 6.5). After a few minutes, the colloidal precipitate was removed and the filtrate was allowed to stand for an hour in an ice-box. Fine powdery crystals (300 mg.) were obtained, m.p. 205–207°C with decomposition. (in 57 % yield) $[\alpha]_D^{13} = +26.43$ (30 mg./cc. water)

Anal. Found: C, 33.17; H, 4.48; N, 16.88. Calcd. for $C_9H_{15}O_4N_4Br$: C, 33.43; H, 4.33; N, 17.33 %.

L-Seryl-L-histidyl-L-leucine. — *N*-Cbzo-*O*-Bz-L-seryl-L-histidyl-L-leucine (0.5 g.) was dissolved in 5 cc. of dioxane and dry hydrogen bromide was passed to produce about 25 % solution. After standing for an hour at room temperature, dihydrobromide of free peptide (0.4 g.) was precipitated on addition of dry ether. The precipitated dihydrobromide was dissolved in 3 cc. of absolute ethanol, followed by addition of 0.2 cc. of triethylamine and the mixture was allowed to stand overnight in an ice-box. The free peptide (300 mg., m. p. 215–216°C with decomposition) was obtained. Recrystallization from ethanol containing a small amount of water gave 200 mg. of the pure peptide in 70 % yield, m. p. 230°C with decomposition. $[\alpha]_D^{13} = -25.3$ (10 mg./cc. water)

Anal. Found: C, 49.03; H, 6.90; N, 18.84. Calcd. for $C_{15}H_{25}O_5N_5 \cdot H_2O$: C, 48.22; H, 7.20; N, 18.80 %.

***O*-Bz-DL-serine Ethylester Hydrochloride.**— Ten grams of *O*-Bz-DL-serine was suspended in 100 cc. of absolute ethanol and the solution was saturated with dry hydrogen chloride at 0°C. After kept standing overnight at 5°C the mixture was evaporated in vacuo and this procedure was repeated. The residual crystals were recrystallized twice from ethanol-ether. The yield (11 g.) was about 80 %, m.p. 118°C.

Anal. Found: C, 54.90; H, 6.79; N, 5.59. Calcd. for $C_{12}H_{18}O_3NCl$: C, 55.49; H, 6.98; N, 5.40 %.

Cbzo-glycyl-*O*-Bz-DL-serine Ethylester. — *O*-Bz-DL-serine ethylester was prepared from 5.2 g. of its hydrochloride by the use of ammonia-chloroform solution. This free ester in 30 cc. of chloroform was coupled with 2.9 g. of Cbzo-glycine in 20 cc. of chloroform and 3.1 g. of dicyclohexycarbodiimide in 10 cc. of chloroform. The reaction mixture was allowed to stand overnight, and shaken with 5 cc. of 0.5 N hydrochloric acid to remove the unchanged dicyclohexylcarbodiimide. After filtration of the dicyclohexyl urea produced, the filtrate was washed with dilute acid, alkali and water, and then dried over anhydrous sodium sulfate. The solvent was removed in vacuo. By adding petroleum ether to the residual, 5.1 g. of Cbzo-glycyl-*O*-Bz-DL-serine ester was obtained in 88 % yield, m.p. 80–83°C. Recrystallization from ethylacetate-petroleum ether gave the pure material, m. p. 84°C.

Anal. Found: C, 63.81; H, 6.58; N, 6.84. Calcd. for $C_{22}H_{26}O_6N_2$: C, 63.74; H, 6.32; N, 6.76 %.

Cbzo-glycyl-*O*-Bz-DL-serine Hydrazide. — Into the solution of 2.1 g. of Cbzo-glycyl-*O*-Bz-DL-serine ethyl ester in 40 cc. of ethanol was added 0.3 g. of hydrazine hydrate and the mixture was allowed to stand overnight at room temperature. The crystals produced were collected and washed with ethanol. Recrystallization from hot ethanol gave 1.8 g. of hydrazide in 90 % of theory, m.p. 188–189°C.

Anal. Found: C, 60.22; H, 5.50; N, 14.08. Calcd. for $C_{20}H_{24}O_5N_4$: C, 59.99; H, 6.04; N, 13.99 %.

***O*-Bz-L-serine Methylester Hydrochloride.** —From 5.7 g. of *O*-Bz-L-serine, 7.2 g. of its methyl ester was obtained by the same method as in the case of O-Bz-L-serine ethyl ester. Recrystallization from methanol-ether gave 6.5 g. of pure crystals in 90 % yield, m.p. 169°C. $[\alpha]_D^{13} = -2.7$ (30 mg./cc. water)

Anal. Found: C, 53.76; H, 6.20; N, 5.52. Calcd. for $C_{11}H_{16}O_3NCl$: C, 53.77; H, 6.54; N, 5.70 %.

Summary

L-Seryl-L-histidine and L-seryl-L-histidyl-L-leucine have been synthesized in good yields by Sheehan's dicyclohexylcarbodiimide method. In the removal of protecting groups of *O*-Bz-serine derivatives by Ben-Ishai's method, dioxane must be used as the solvent in place of glacial acetic acid. *O*-Bz-L- and DL-serine ester was synthesized the mild esterification condition.

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